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ORIGINAL ARTICLE

The association between Nd:YAG laser and desensitizing dentifrices for the treatment of dentin hypersensitivity

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Abstract This study aims to evaluate the association between Nd:YAG laser (with and without a photoabsorber) and two desensitizing dentifrices containing 15% NovaMin or 8% arginine, as potential treatments for dentin hypersensitivity (DH). DH was simulated by EDTA application for 2 min. Specimens were then analyzed with an environmental scanning electron microscope (ESEM) to ensure open dentin tubules (ODT), counted by using ImageJ software. Specimens were randomized into eight groups ($n = 10$): Laser (L), Laser+Photoabsorber (LP), Arginine (A), Arginine+Laser (AL), Arginine+Laser+Photoabsorber (ALP), NovaMin (N), NovaMin+Laser (NL), and NovaMin+Laser+Photoabsorber (NLP). Laser irradiation was performed with 1 W, 100 mJ, 10 Hz, $\cong 85$ J/cm^2; 4 irradiations of 10 s each, with 10 s intervals between them. After treatment, specimens were again analyzed by ESEM and submitted to erosive/abrasive cycling for 5 days. A final ESEM analysis was performed. Data were analyzed with two-way repeated measure ANOVA and Tukey tests ($\alpha = 0.05$). After treatment, groups N, NL, and NLP presented the lower number of ODT, but they did not different from LP, ALP, and AL. Group A presented the highest number of ODT and it did not differ from group L. Groups L, AL, ALP, and LP presented intermediate results, without differing from each other. After cycling, group A presented the highest number of ODT and did not differ significantly from the other groups, except NLP. None of the associations tested presented better tubule occlusion than NovaMin by itself. Arginine was the only treatment that presented improved tubule occlusion when associated with Nd:YAG laser.

Keywords Dentin · Dentin hypersensitivity · Laser · Nd:YAG · Dentifrices · Desensitizers

Introduction

Dentin hypersensitivity (DH), a common condition among patients worldwide, often appears in the cervical region of the tooth, where enamel loss and/or gingival recession can cause exposure of the dentin tubules [1–4]. DH is characterized as a stimulus-induced, localized, and short-duration pain (that stops after the stimulus is removed), which cannot be associated with any other pathology or dental defect [5–7]. Hypersensitive dentin has been shown to present a significant increase in the number of exposed dentin tubules per area (up to 8×), in addition to a larger tubule diameter (up to 2×), when compared with non-sensitive dentin [8, 9].

To explain the pain mechanism in DH, the theory proposed by Brännström is the most widely accepted. According to this theory, a stimulus-induced shift in the movement of the intratubular fluid (either inward or outward) can activate the pulpal nociceptors, resulting in pain [10, 11]. Taking this theory into account, the main strategy for the treatment of DH consists of sealing the dentinal tubules, thus preventing fluid flow movement. This can be performed with restorations, desensitizing agents, adhesive systems, sodium fluoride, and high-power laser irradiation [12–15]. Nowadays, there are a large number of commercially available desensitizing agents

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with different active agents for DH, such as arginine plus calcium carbonate and calcium sodium phosphosilicate (described as the NovaMin technology) that have presented promising results in the treatment of DH [13, 16].

Arginine is a natural amino acid, present in human saliva, which can easily bind to calcium carbonate, facilitating its adherence to the dentin, where it can form calcium plugs inside the dentinal tubules [16, 17]. Whereas, NovaMin is the trade name for a calcium sodium phosphosilicate-based bioactive glass. The reaction between NovaMin and saliva releases Na^+, causing an increase in salivary pH, leading to the calcium (Ca^{2+}) and phosphate (PO_4^3) ions precipitating in the form of hydroxycarbonate apatite (HCA) capable of sealing opened dentinal tubules [18–20].

High-power lasers, such as the Nd:YAG laser, are a contemporary alternative for treating DH [21]. Nd:YAG laser has the ability to seal dentinal tubules to a depth of approximately 4 μm [22]. This is achieved by heating the dentin until it melts and solidifies again. Nevertheless, the correct protocols must be used to avoid pulp injuries or cracks in the dental tissues [22–24]. The Nd:YAG laser wavelength has an absorption peak with dark pigments. Previous studies have shown that the association between Nd:YAG laser and a dark photoabsorber in dentin caused an additional increase in the surface temperature, protecting the pulp from higher temperatures and improving dentin sealing [25–27].

Data about the association between high-power lasers and desensitizing dentifrices are scarce. According to two previous investigations, associating Nd:YAG laser with a NovaMin paste did result in more tubule occlusion than the laser alone [28, 29]. To the author's knowledge, there is no information about the combined use of Nd:YAG laser and arginine.

In view of the foregoing, the objectives of this study were to evaluate the association between Nd:YAG laser (with and without a photoabsorber) and two desensitizing dentifrices (containing 15% NovaMin or 8% Arginine) as potential treatments for dentin hypersensitivity (DH), and to verify whether the treatments would sustain tubule occlusion after 5 days of erosive/abrasive challenges.

Thus, the null hypotheses of the study were as follows: 1. The association between laser and the desensitizing agents (Arginine and NovaMin) would not promote better tubule obliteration when compared with these treatments used separately; 2. Treatment results would not differ after erosive/abrasive cycling.

Materials and methods

Preparation of samples

Eighty sound human third molars were collected from the Tooth Bank of the University of São Paulo, School of Dentistry, after approval from the Research Ethics Committee of the same institution (Protocol 1.584.970). Teeth were cleaned and their roots were separated from the crowns by using a water-cooled diamond disk (KG Sorensen; Barueri, São Paulo, Brazil). From the roots, dentin slabs (4 mm × 4 mm × 2 mm) were cut, ground flat, and polished with water-cooled abrasive discs (1200-, 2400-, and 4000-grit). Between each polishing step, the specimens were sonicated in distilled water (Digital Ultrasonic Cleaner CD-4820, Kondortech, Sao Carlos, Brazil) for 8 min, to remove any debris. To simulate hypersensitive dentin, the specimens were immersed in 17.5% EDTA solution for 2 min, to remove the smear layer and open the dentin tubules [30].

Environmental scanning electron microscopy evaluation

After EDTA application, all specimens were analyzed by environmental scanning electron microscopy (ESEM) (3) to qualitatively and quantitatively verify the number of open dentin tubules. Representative micrographs were taken at ×2000, by using Analy observation conditions, at the center, northwest, and southeast of each specimen. No sample preparation was required. All specimens were re-evaluated after treatments and cycling. In the qualitative assessment, the surface characteristics of micrographs were evaluated and checked for patency and occlusion of the dentinal tubules. In the quantitative assessment, the number of open dentin tubules was counted with an image analysis software program, ImageJ (NIH) [31].

Treatments

After opening the dentinal tubules to simulate hypersensitive dentin (control time interval) and perform the first tubule counting, the specimens were randomly allocated into eight experimental groups ($n = 10$), according to their respective treatment (Table 1). All laser groups (L, LP, AL, ALP, NL, and NLP) were treated with a Nd:YAG laser (Power LaserTM ST6, Lares Research, Chico, CA) used in contact mode, focused, and in a perpendicular direction, with the following parameters: 1.0 W power, 10 Hz repetition rate, energy of 100 mJ, and an energy density of ≅85 J/cm² per pulse. A 400-μm quartz fiber was used in the x- and y-axis directions. This procedure was performed in four 10 s irradiations (two in each direction) with an interval of 10 s between the irradiations to allow thermal relaxation of the dentin tissue. All groups with photoabsorber (PL, APL, and NPL) received a thin layer of a solution composed of triturated coal diluted in equal parts of deionized water and 99% ethanol after the treatments with dentifrices were performed and before the laser application. Group Arginine (A) was treated with Colgate Sensitive Pro-Relief, containing 8% arginine and 1.450 ppm F, as sodium monofluorophosphate (MFP). The dentifrice was

Table 1 Experimental groups and treatments

Groups	Treatments
Laser (L)	Nd:YAG laser; 1 W, 10Hz, 100 mJ, 85 J/cm^2
Laser+Photosensitizer (LP)	Coal + Water + Ethanol + Nd:YAG laser; 1 W, 10Hz, 100 mJ, 85 J/cm^2
Arginine (A)	1 min prophylaxis with Colgate Sensitive Pro-Relief
Arginine+Laser (AL)	1 min prophylaxis with Colgate Sensitive Pro-Relief+Nd:YAG laser; 1 W, 10Hz, 100 mJ, 85 J/cm^2
Arginine+Laser+Photosensitizer (ALP)	1 min prophylaxis with Colgate Sensitive Pro-Relief+Coal+Water+ Ethanol+Nd:YAG laser; 1 W, 10Hz, 100 mJ, 85 J/cm^2
NovaMin (N)	1 min prophylaxis with NuPro Extra Care
NovaMin+Laser (NL)	1 min prophylaxis with NuPro Extra Care+Nd:YAG laser; 1 W, 10Hz, 100 mJ, 85 J/cm^2
NovaMin+Laser Photosensitizer (NLP)	1 min prophylaxis with NuPro Extra Care+Coal+Water+Ethanol+Nd: YAG laser; 1 W, 10Hz, 100 mJ, 85 J/cm^2

applied with a rubber cup mounted on low speed hand piece, for 1 min. Group NovaMin (N) was treated with NuPro® Extra Care Prophy paste, containing 15% NovaMin and 12.210 ppm F, as sodium fluoride. This dentifrice was applied for 1 min as prophylaxis, in accordance with the manufacturer's instructions.

Erosive/abrasive challenges

A modified 5-day erosion-abrasion-remineralization model (Scaramucci et al. 2013) (Table 2) was used [32]. Erosive challenges were performed with 0.3% citric acid solution (pH = 2.4, natural pH). The specimens were immersed in citric acid for 5 min, four times a day, without agitation and at room temperature. After the erosion episode, specimens were rinsed with distilled water and gently dried with absorbent paper, followed by exposure to artificial saliva (1.649 mmol/l $CaCl_2 \cdot H_2O$; 5.715 mmol/l KH_2PO_4; 8.627 mmol/l KCl; 2.950 mmol/l NaCl g/l; 92 mmol/l Tris buffer; pH adjusted

Table 2 Daily cycling sequence

Procedures	Solutions and times
Erosive challenge	Citric acid 0.3% (2 min)
Remineralization	Artificial saliva (30 min)
Brushing	15 s (2 min suspension exposure)
Remineralization	Artificial saliva (30 min)
Erosive challenge	Citric Acid 0.3% (2 min)
Remineralization	Artificial saliva (60 min)
Erosive challenge	Citric acid 0.3% (2 min)
Remineralization	Artificial saliva (60 min)
Erosive challenge	Citric acid 0.3% (2 min)
Remineralization	Artificial saliva (30 min)
Brushing	15 s (2 min suspension exposure)
Remineralization	Artificial saliva (120 min)

to 7 with HCl) for 30 min, before the abrasive challenge; or 60 min, before another erosion episode.

Toothbrushing was performed twice a day for 15 s, in the middle of the first and last remineralization periods, with electric brushes (Oral B Professional Care 3000f), equipped with a pressure alert feature that signaled when the pressure had reached the value of 2.5 N. The toothbrush head was positioned parallel to the surface of the specimens until the pressure alert was turned on. Slurry of standard 1450 ppm F$^-$, as NaF, toothpaste (Colgate Total 12 Clean Mint) was prepared with distilled water (1:3 *w/w*) and used for brushing. Total exposure time of the specimens to the dentifrice slurries, in each brushing episode, was 2 min. A single operator performed the toothbrushing procedures. Overnight, the specimens were stored in a humid environment, at 4 °C.

Statistical analysis

Open dentin tubule data were analyzed for normal distribution and homoscedasticity with Shapiro-Wilks and Brown-Forsythe tests, respectively. Comparisons among groups were performed with two-way repeated measures ANOVA and Tukey tests. The software Sigma plot, version 12, was used for all calculations. The significance level was set at 5%.

Results

Two-way repeated measures ANOVA showed significant differences among the levels of the factors *treatment* ($p < 0.001$), *time* ($p < 0.001$), and in the interaction between them ($p < 0.001$).

Regarding the factor *treatment*, after the control time interval, there were no significant differences in the number of open dentin tubules among groups ($p > 0.05$). After treatments, Arginine presented the highest number of open dentin tubules, differing significantly from all groups, except group

Laser. Group Laser did not differ significantly from Arginine+Laser, Arginine+Laser+Photosensitizer, and Laser+Photosensitizer. Treatments presenting the lowest number of open dentin tubules were NovaMin, NovaMin+Laser, and NovaMin+Laser+Photoabsorber, but they did not differ from Laser+Photoabsorber, Arginine+Laser+Photoabsorber, and Arginine+Laser.

After cycling, Arginine presented the highest number of open tubules, although it did not differ significantly from the other groups, except from group NovaMin+Laser+Photoabsorber, which was capable of maintaining the lowest number of open dentin tubules.

Relative to experimental times, all groups with laser treatment, except for Laser+Photoabsorber, presented significant differences among them. In the control time interval, the number of open dentin tubules was the highest, followed by cycling, and then by DH treatments. For Laser+Photoabsorber and NovaMin, there were no significant differences between the times after EDTA application and cycling, but both significantly differed from DH treatment. For group Arginine, no significant differences were observed between DH treatments and cycling; however, both differed from the control.

Means (SD) of open dentin tubules according to each DH treatment in all experimental time intervals are shown in Table 3. Images of each group in each time interval are shown in Figs. 1, 2, and 3. An example of ImageJ software analysis of open dentinal tubules may be visualized in Fig. 4.

Discussion

In this study, desensitizer agents such as Arginine, NovaMin, Nd:YAG laser, and the association between them showed they were capable of obliterating dentinal tubules immediately after their application, making them suitable approaches to the treatment of DH.

NovaMin was the treatment that provided the most satisfactory results for dentin tubule occlusion, irrespective of its association with other treatments. In this study, NovaMin efficiency may be explained by the action of its active ingredient, a bioactive glass composed of Ca, P, Na, Si, O, and a high NaF concentration (12.210 ppm F) that may have induced the formation of an apatite-like layer on the dentin surface. The immediately positive effect of NovaMin on tubule occlusion is in agreement with previous in vitro and in situ studies [33, 34] and it is also corroborated by a clinical investigations that reported a significantly reduction in hypersensitivity over a period of 30 days [35–38].

Arginine was the least effective treatment observed in this study. This might have occurred because Colgate Sensitive Pro-Relief has a lower fluoride concentration (1.440 ppm F) when compared with NuPro, the product with NovaMin (12.210 ppm F). Furthermore, in the arginine paste, fluoride was presented as sodium monofluorophosphate (NaMFP), whereas in NuPro, fluoride was derived from NaF. NaMFP needs be hydrolyzed by salivary alkaline phosphatase to produce free fluoride ions [39]. Since artificial saliva was used in this study to emphasize the role of the desensitizing components, the enzymatic and microbiological effects of human saliva could not be expected. This may have contributed to the lower efficacy of the arginine paste, as has previously been suggested [30, 40]. Considering that some clinical studies observed a positive effect of arginine in reducing dentin hypersensitivity, caution should be taken when analyzing the results of this investigation [41–43].

Although Nd:YAG laser irradiation has shown excellent ability to seal dentinal tubules, it was unable to create homogeneous melting across the entire dentin surface. This may be related to the manual irradiation performed, instead of using a scanner table. Untreated areas could be observed in the micrographs, and they may have contributed to the lower effectiveness of this treatment when compared with NovaMin paste. Manual simulation was chosen because it simulated the clinical situation more closely, and it was already shown that clinically, Nd:YAG is an effective tool in reducing DH [44–46].

With the association of the Nd:YAG laser and a photosensitizer, the authors observed no superior results when compared with the use of laser alone. This might have occurred because of the protocol with high power (1 W) used, which has been tested in the past [24, 28–30], was thus able to reach melting point without the help of the dark pigment. Near

Table 3 Means and standard deviation of opened dentin tubules according to each DH treatment and times. Different uppercase letters denote significant difference among groups within experimental time ($p < 0.05$). Different lowercase letters imply significant difference among experimental times within groups ($p < 0.05$)

	Control	Treatment	Cycling
L (Laser)	111(36) Aa	56(25) ABc	87(24) ABb
LP (Laser+Photoabsorber)	89(11) Aa	24(12) BCb	84(15) ABa
A (Arginine)	126(30) Aa	79(26) Ab	99(28) Ab
AL (Arginine+Laser)	112(44) Aa	38(17) BCc	82(33) ABb
ALP (Arginine+Laser+Photoabsorber)	109(32) Aa	35(17) BCc	80(32) ABb
N (NovaMin)	95(21) Aa	20(17) Cb	90(28) ABa
NL (NovaMin+Laser)	108(29) Aa	23(18) Cc	83(38) ABb
NLP (NovaMin+Laser+Photoabsorber)	111(44) Aa	4(6) Cc	54(20) Bb

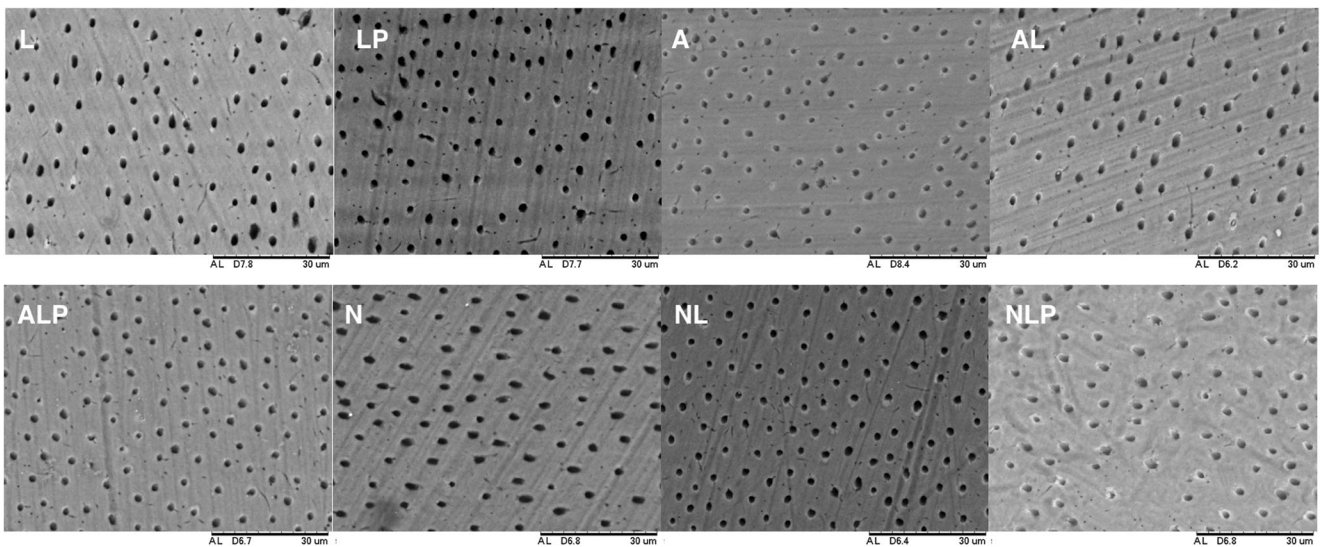**Fig. 1** SEM images with ×2000 magnification of each group in the control time

infrared lasers, such as diode and Nd:YAG lasers, are mostly absorbed by pigments, such as hemoglobin and melanin. Furthermore, they penetrate into the tissues more deeply than the infrared wavelengths of the erbium and CO_2 lasers. To protect and to collimate the laser light, the use of black photoabsorbers has been suggested. Whereas, as clinically observed, staining of the exposed dentin has been a common and undesirable consequence, which may restrict its use in aesthetic areas. Nevertheless, without the use of the photoabsorber, the thermal effects produced by Nd:YAG laser on the pulp are still unclear [47, 48].

Regarding the association of treatments, the only combination that presented better tubule occlusion than the paste used alone was the association between arginine and laser, thus rejecting the first null hypothesis. This improvement could be explained by the fact that Arginine alone presented no great capacity for

tubular occlusion, leaving a large number of open tubules behind. The laser then provided action complementary to that of Arginine, by means of melting and resolidification in the areas where the tubules remained open. As NovaMin alone was capable of occluding a large number of tubules, the melting did not show a significant effect because it was performed on the tubules that had already been occluded by the prophylaxis paste.

When NovaMin was used in association with Nd:YAG laser, the authors observed no further tubule occlusion. It could be hypothesized that since NovaMin presented great capacity for tubule obliteration, the melting produced by the Nd:YAG laser occurred in tubules that were already occluded. In a previous study, Farmakis et al. (2013) showed better ability of Nd:YAG laser for tubule occlusion when compared with NovaMin alone, but no additional benefits were observed when the laser was associated with NovaMin [29]. Later, these authors
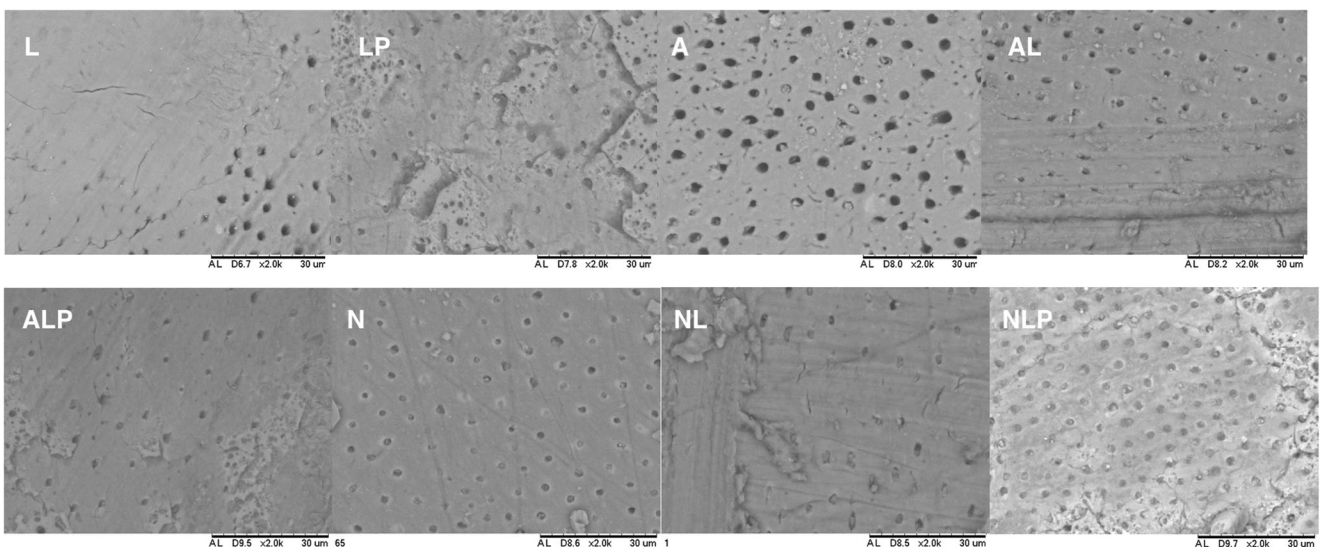

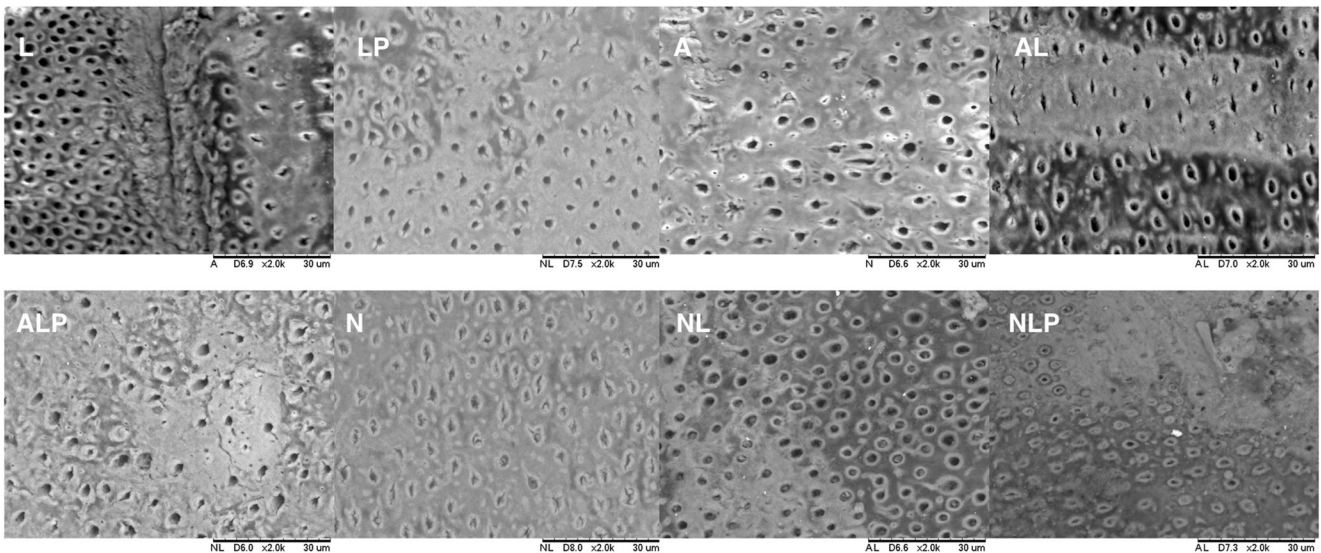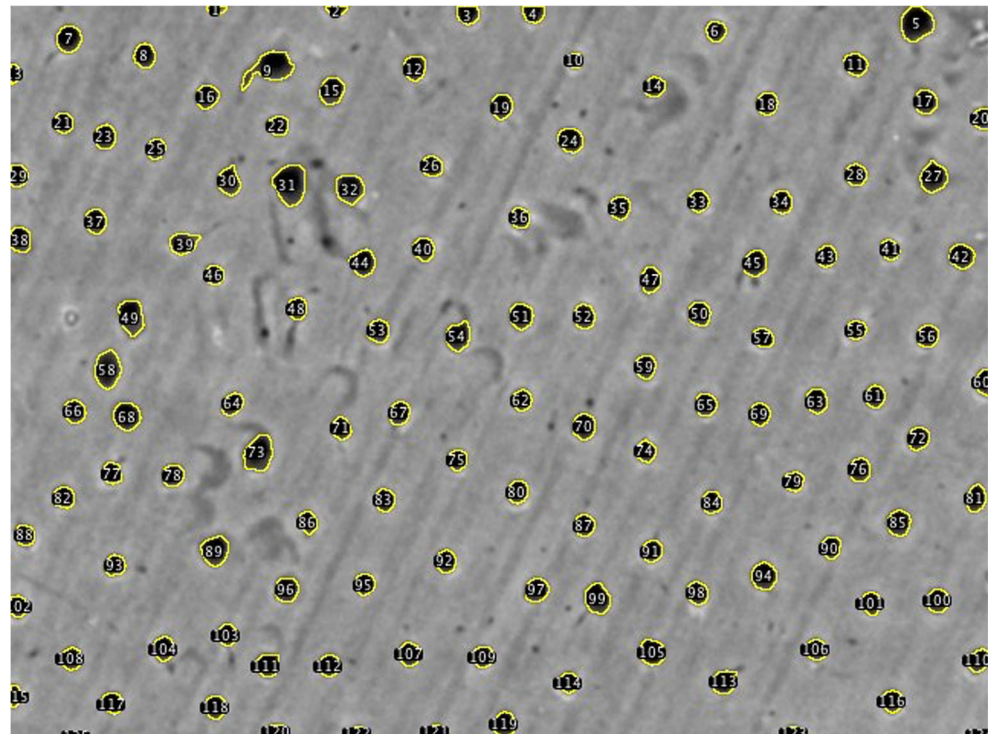**Fig. 2** SEM images with ×2000 magnification of each group after treatment

Fig. 3 SEM images with ×2000 magnification of each group after cycling

demonstrated that when Nd:YAG laser irradiation at 1 W (the same power used in the present study) was used alone or combined with NovaMin, it was a superior method for producing dentinal occlusion, being outstanding as an effective treatment modality for DH [28]. It is worth mentioning that in the above-mentioned study, an over-the-counter NovaMin paste was used for 5 min, directly on the exposed dentin. In the present study, the authors used a prophylaxis paste containing NovaMin, in addition to NaF that was in a higher concentration than that in the over-the-counter paste, which could be the responsible for the difference in the results. Considering the outcomes of the present

investigation, it may be inferred that while the effect of NovaMin paste might be enhanced by the action of Nd:YAG laser, the prophylaxis paste itself had an effective action, which was not further complemented by the laser.

As expected, no desensitizing treatment was able to sustain tubule occlusion after the erosive/abrasive cycling, results similar to those in previous reports [27–32], allowing acceptance of the second null hypothesis of this study. It should, however, be considered that the laser groups presented lower trend towards dentinal tubule re-opening when compared with the pastes used alone. It could be hypothesized that the

Fig. 4 ImageJ count of opened tubules

melting created by Nd:YAG caused structural changes in the substrate, fusing material over the dentin and within the dentin tubules. Whereas, the prophylaxis pastes only created a layer of deposits, which could more easily be removed.

This study used an erosion/abrasion/remineralization cycling model that intended to simulate the clinical situation of individuals suffering from DH, since dietary acids and toothbrushing are known to be capable of opening and enlarging dentin tubules, reducing the effectiveness of DH treatments [27, 49, 50]. The cycling protocol was adapted from Scaramucci et al. (2013) and attempted to simulate the situation of patients with high frequency consumption of acidic beverages [32]. The literature has reported that in the mouth, the maximum time during which the pH remains low is about 2 min, and extrapolation of this condition may modify the eroded surface to an unrealistic state [51, 52]. Toothbrushing abrasion occurred twice a day, in an endeavor to simulate a realistic daily oral hygiene habit [53]. This was performed with an electrical toothbrush, fixed in a specific device that standardized the brush movement over the specimen and controlled the brushing force at 2.5 N, which is within the range of force recommended for erosion-abrasion studies [54, 55].

Colgate Total 12 Mint Clean was chosen for toothbrushing because it is a regular 1450 ppm F (as NaF) toothpaste, without any desensitizing agent [40]. The slurry used was prepared with distilled water instead of artificial saliva. Although artificial saliva has calcium in its composition, and this could enhance the action of the desensitizing agents tested, distilled water was chosen to avoid any possible reaction of the agents during mixing, before they reached the dentin specimens. Another limitation of the present study was that it was performed in vitro, without the presence of the pulpal pressure. In this sense, care should be taken when extrapolating the findings of this study to the clinical scenario.

Conclusion

Considering the limitations of this in vitro investigation, NovaMin prophylaxis paste presented promising results concerning the obliteration of dentin tubules immediately after treatment. None of the treatments were capable of preventing the re-opening of the tubules after erosive/abrasive challenges.

Compliance with ethical standards

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